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Follow-up standard agglutination and 2-mercaptoethanol tests in 175 clinically cured cases of human brucellosis

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ABSTRACT

Background: The standard agglutination (SAT) and 2-mercaptoethanol (2-ME) tests are usually used in the follow-up of treated cases of human brucellosis. The purpose of this study was to monitor the levels of these tests, two years after clinical cure in cases of brucellosis.
Methods: From April 2003 to September 2008, 175 clinically cured cases of brucellosis (103 males, 72 females) were evaluated. Diagnosis of brucellosis was established with a SAT of ≥1:320 and a 2-ME of ≥1:80, with clinical symptoms and signs compatible with brucellosis. SAT and 2-ME were retested at the end of therapy and at 3-monthly intervals for two years. Serologic cure was considered in the event of a SAT titer decrease to ≤1:160 or a 2-ME decrease to < 1:80.
Results: The mean age of study patients was 31 ± 13.5 years. At 6, 12, 18, and 24 months after treatment, SAT titers ≥1:320 were seen in 41 (23.4%), 22 (12.6%), 7 (4%), and 6 (3.4%) cases, respectively, whereas 2-ME titers ≥1:80 were seen in 51 (29.1%), 24 (13.7%), 12 (6.9%), and 8 (4.6%) cases, respectively. The probability of serologic cure for patients with SAT titers ≤1:640 was higher than for those >1:640 (95% confidence interval (CI) 2.5–3.47, $p = 0.023$). The probability of serologic cure for patients with 2-ME titers ≤1:320 was higher than for those >1:320 (95% CI 2.48–3.5, $p = 0.04$).
Conclusions: SAT and 2-ME may be found in significant titers in less than 5% of clinically treated cases after two years. Serologic cure for both tests with lower titers were higher than with higher titers.

1. Introduction

Brucellosis is an important public health problem in many countries throughout the world, including Iran.[1,2] The definitive diagnosis of patients with brucellosis is isolation of the organism from the blood or other body fluids, however isolation of the organism is achieved in 10–70%.[3,4] A standard agglutination test (SAT) titer ≥1:320 and 2-mercaptoethanol (2-ME) titer ≥1:80 in patients with compatible clinical findings are the most important methods for diagnosing brucellosis in developing countries.

In endemic areas, >90% of patients with acute bacteremia have SAT titers of at least 1:320.[5,6] The SAT measures the total amount of agglutinating antibodies of IgM and IgG, and the 2-ME test measures IgG antibodies and strongly indicates active infection. A rapid fall in the level of IgG antibodies is prognostic of successful therapy.[7] Relapse may occur with any therapy regimen.[8–13] Thus patients should ideally be followed clinically and serologically for up to two years to detect any case of relapse. IgG antibody levels detected by SAT and variants of this test can remain in the diagnostic range for more than two years after successful treatment.[4] Thus it is warranted to caution against treating positive titers in those asymptomatic patients previously treated for brucellosis. A resurgence in antibody titers most likely indicates relapse or reinfection.[14] A fall in the 2-ME titer reflects a satisfactory response to treatment. It indicates a favorable response to antibiotic therapy and that no further antibiotic treatment is required.[7] Since reports of the serologic follow-up of successfully treated cases are limited in the medical literature, the purpose of this study was to assess the follow-up titers of SAT and 2-ME in clinically treated cases of brucellosis.

2. Methods

From April 2003 to September 2008, 175 clinically cured cases of brucellosis, followed for two years at the Department of Infectious Diseases of Babol Medical University in Iran, were studied. These cases were selected from patients who were treated either with streptomycin 1 g IM for two weeks and doxycycline

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100 mg twice daily for 45 days (99 cases) or with gentamicin 5 mg/kg/day for seven days plus the same dose of doxycycline for 45 days (105 cases). Brucellosis in these patients was diagnosed when they had a SAT titer ≥1:320 and 2-ME titer ≥1:80 with clinical signs and symptoms compatible with brucellosis.

In treated cases, 'clinical cure' was defined as resolution of all clinical symptoms and signs of brucellosis after the end of treatment. The aim of this study was to determine serologic cure (SAT or 2-ME) in cured cases of brucellosis during a two-year follow-up. Falls in titer of SAT or 2-ME over time are prognostic of a good response to therapy.[1,3,13] Therapeutic failure due to lack of efficacy was defined by symptoms or signs of the disease that persisted at the end of treatment as judged clinically. Relapse was defined to have occurred when clinical symptoms and signs of brucellosis reappeared after completion of therapy and reduced titers of SAT or 2-ME increased again.[3] Cases of initial therapeutic failure and relapse and those who were not followed for two years were excluded from the study. SAT and 2-ME titers were recorded at baseline, after treatment, and at three-monthly intervals for two years. SAT and 2-ME test antigen was obtained from the Institute Pasteur, Iran and tests were interpreted according to routine methods and criteria. SAT and 2-ME were performed in glass reaction tubes and the agglutination was observed under light in each tube. Serologic cure in clinically cured subjects was determined when the titers of SAT and 2-ME decreased to less than 1:320 and 1:80, respectively. The study was approved by the Infectious Diseases Research Center of the Babol Medical University.

Data were analyzed using SPSS version 15 (SPSS Inc., Chicago, IL, USA). The reductions in SAT and 2-ME titers over time were determined in all cases. The log rank test was used to compare SAT cure in patients with titers ≤1:640 and >1:640 as well as serologic cure of 2-ME ≤1:320 and >1:320. Times for reduction of SAT and 2-ME were depicted using Kaplan–Meier graphs. Ninety-five percent confidence intervals (CIs) were calculated when appropriate. Differences with a p-value of < 0.05 were considered significant.

3. Results

In the gentamicin and doxycycline treated patients, seven cases had initial therapeutic failure or relapse and six patients did not participate in our follow-up and were excluded from the study. In the streptomycin and doxycycline treated patients, nine cases had initial therapeutic failure or relapse and seven subjects did not participate in our follow-up period and were excluded from the study. Hence, a total of 16 (7.8%) cases were not clinically or serologically responsive and 13 (6.4%) did not participate in our follow-up study. Therefore, 175 clinically cured cases in both treated groups (103 males and 72 females), with a mean age of 31 ± 13.5 years, who were followed for two years, were evaluated.

SAT titers of between 1:320 and 1:1280 were seen in 166 (94.9%) cases before the initiation of therapy. After treatment, SAT titers <1:320 were seen in 42 (24%) of the cases. Despite clinical cure, SAT and 2-ME titers were increased compared to baseline levels in six (3.4%) and 10 (5.7%) cases, respectively, although in these cases, the levels of SAT or 2-ME fell below the baseline levels after three months. At six and 12 months after treatment, SAT <1:320 was seen in 134 (76.6%) and 153 (87.4%) cases, respectively. Eighteen and 24 months after treatment SAT titers ≥1:320 were seen in seven (4%) and six (3.4%) patients, respectively (Table 1). The probability of serologic cure of SAT for patients with titers ≤1:640 was higher than for those >1:640 (95% CI 2.5–3.47, $p = 0.023$) (Figure 1).

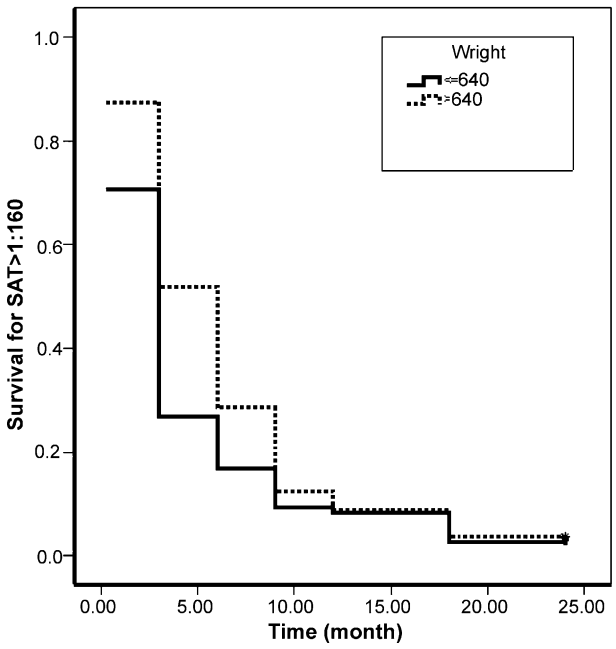

Figure 1. Probability for cure with SAT titers >1:640 and ≤1:640 in patients with brucellosis who were successfully cured. The log rank test shows that the difference was significant ($p = 0.023$).

At baseline, 2-ME titers ≥1:160 were seen in 127 (72.6%) cases (Table 2). After six and 12 months of treatment, 2-ME titers ≥1:80 were seen in 51 (29.1%) and 24 (13.7%) cases, respectively. Eighteen and 24 months after treatment, 2-ME ≥1:80 was seen in 12 (6.9%) and eight (4.6%) cases, respectively (Table 2). The probability of serologic cure for the 2-ME test in patients with titers ≤1:320 was higher than for those >1:320 (95% CI 2.48–3.5, $p = 0.04$) (Figure 2).

4. Discussion

In this study, we found that SAT serologic cure increased from 42 (24%) patients at the end of treatment to 153 (87.4%) one year after treatment. This rate increased to 96.6% after two years. The cure rate for the 2-ME test was 17.1% at the end of treatment and was 86.3% after one year. Only eight (4.6%) cases had significant titers for 2-ME after two years. Serologic cure for both tests in patients with lower titers were higher than with higher titers.

Table 1
SAT titers at baseline, at the end of therapy, and at follow-up over two years, for 175 treated cases of brucellosis

SAT	<1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
At baseline	-	-	-	69 (39.4)	50 (28.6)	47 (26.9)	8 (4.6)	1 (0.6)
At end of treatment	6 (3.4)	14 (8)	22 (12.6)	59 (33.7)	38 (21.7)	30 (17.1)	5 (2.9)	1 (0.6)
3 months later	47 (26.9)	33 (18.9)	33 (18.9)	24 (13.7)	30 (17.1)	5 (2.9)	2 (1.1)	1 (0.6)
6 months later	82 (46.9)	32 (18.3)	20 (11.4)	23 (13.1)	12 (6.9)	6 (3.4)	0 (0)	0 (0)
12 months later	119 (68)	23 (13.1)	11 (6.3)	15 (8.6)	6 (3.4)	1 (0.6)	0 (0)	0 (0)
18 months later	150 (85.7)	8 (4.6)	10 (5.7)	3 (1.7)	4 (2.3)	0 (0)	0 (0)	0 (0)
24 months later	154 (88)	10 (5.7)	5 (2.9)	4 (2.3)	2 (1.1)	0 (0)	0 (0)	0 (0)

Results are n (%).
SAT, standard agglutination test.

Table 2
2-ME titers at baseline, at the end of therapy, and at follow-up over two years, for 175 treated cases of brucellosis

2-ME	0	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
At the baseline	-	-	-	48 (27.4)	57 (32.6)	42 (24)	20 (11.4)	8 (4.6)
End of therapy	9 (5.1)	5 (2.9)	16 (9.1)	46 (26.3)	56 (32)	27 (15.4)	11 (6.3)	5 (2.9)
3 months later	48 (27.4)	16 (9.1)	32 (18.3)	33 (18.9)	31 (17.7)	13 (7.4)	2 (1.1)	0 (0)
6 months later	85 (48.6)	12 (6.9)	27 (15.4)	31 (17.7)	12 (6.9)	6 (3.4)	2 (1.1)	0 (0)
12 months later	124 (70.9)	6 (3.4)	21 (12)	15 (8.6)	5 (2.9)	3 (1.7)	1 (0.6)	0 (0)
18 months later	152 (86.9)	3 (1.7)	8 (4.6)	8 (4.6)	1 (0.6)	2 (1.1)	1 (0.6)	0 (0)
24 months later	152 (86.9)	6 (3.4)	9 (5.1)	5 (2.9)	1 (0.6)	2 (1.1)	0 (0)	0 (0)

Results are *n* (%).
2-ME, 2-mercaptoethanol.

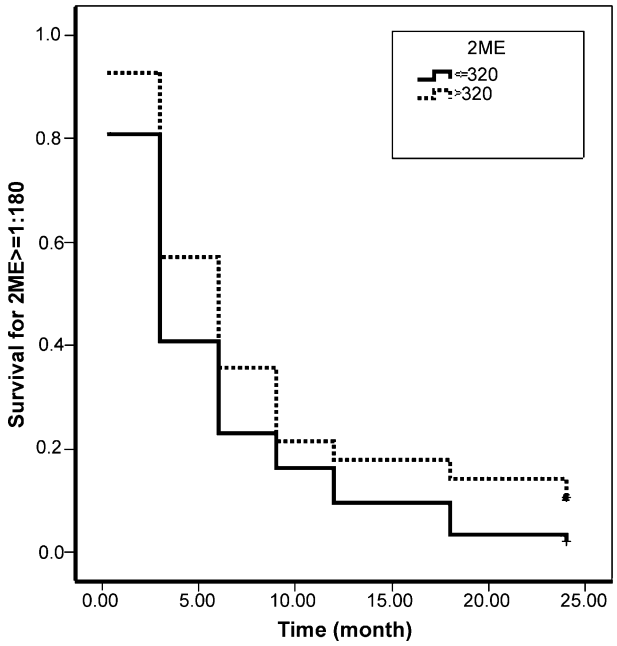

Figure 2. Probability for serologic cure with 2-ME titers >1:320 and ≤1:320 in patients with brucellosis who were successfully cured. The log rank test shows that the difference was significant ($p = 0.04$).

These findings suggest that clinically treated cases with SAT ≤1:640 or 2-ME ≤1:320 at the time of diagnosis lost their specific antibodies rapidly. Thus, patients with higher titers of SAT or 2-ME at baseline may have significant titers of both tests after early post-treatment follow-up. On the other hand, we found that SAT and 2-ME titers increased compared to baseline levels in six (3.4%) and 10 (5.7%) cases, respectively, but the titers fell below baseline levels after three months of post-treatment follow-up. These findings emphasize that serologic titers should be interpreted with caution in clinically cured cases, especially in early post-treatment follow-up.

Serologic tests are not only the most commonly used tests for the diagnosis of brucellosis in endemic regions, but these tests are also useful for follow-up of treated cases in order to determine relapse. Most relapses occur within six months of cessation of therapy.[15] With recovery, titers of IgG and IgM antibodies slowly decline; large numbers of cases do not have significant titers for both antibodies after one year, as we have shown in this study.[16,17] Almuneef and Memish followed 116 clinically cured cases of acute brucellosis in Saudi Arabia and found that 28.6% of their cases had significant titers of SAT after two years. They also found that older age, male gender, and patients treated with fewer than three antibiotics were more likely to have persistently high Brucella antibodies. They showed that a doxycycline-containing regimen of therapy was associated with serologic cure.[18] Buchanan and Faber showed that the titers remained positive in the SAT test much longer than in the 2-ME test and 48% of their 92 cases had SAT titers of ≥1:160, 1.5 years after treatment was begun. However, regarding the 2-ME test, the number of patients with titers of ≥1:160 at 6, 9, 12, and 18 months after illness were 22 (24%), 12 (13%), 8 (9%), and 4 (4%), respectively.[7] The differences in the fall of SAT titers in the two above studies compared to our study may be related to the high titers of SAT in their cases at the time of diagnosis and treatment. On the other hand, the therapy regimens that were used in our study may have influenced the rapid fall in titers of SAT in our cases. Regarding falling titers of 2-ME, our results are in agreement with the findings of Buchanan and Faber and Mantur et al.[7,19] A fall in the 2-ME titer reflects a satisfactory response to treatment. It indicates a favorable response to antibiotic therapy and that no further antibiotic treatment is required.[20]

Recently, investigators have shown Brucella DNA in the sera of a significant number of successfully treated cases who had remained clinically healthy for prolonged follow-up periods, without finding any organism. They concluded that clinical response may not be equivalent to pathogen eradication in brucellosis patients.[21,22] This question is particularly important in the brucellosis endemic regions. On the other hand, asymptomatic bacteriologic relapse is a problem for clinicians working in underdeveloped nations. We believe that until the answers to these questions are found, clinical and serologic follow-up may be the best way to monitor these cases. Thus, caution is warranted against treating positive titers per se to make them disappear in those asymptomatic patients previously treated for brucellosis. The availability of these tests is useful for the diagnosis of human brucellosis, especially in developing countries where most of the modern facilities are not available. The results of this study show that SAT and 2-ME may occur in significant titers in less than 5% of clinically cured cases after two years. Serologic cure for both tests with lower titers were higher than with higher titers.

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Conflict of interest: No conflict of interest to declare.

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